

Comparison of genetic maps for two related pea populations (*Pisum sativum* L.)

Gawłowska, M., Świącicki, W. and Wolko, B.

Inst. of Plant Genet.
Polish Acad. of Sci., Poznań, Poland

Introduction

Genetic maps can serve many plant breeding purposes, such as tagging single genes or permitting the localization of quantitative trait loci (QTL). Such associations between markers and traits are useful for following the trait in the segregating progeny. However, it is not possible to create a map for each breeding population. The most valuable map is thus one with markers transferable to populations other than the mapping population. Here we compare two linkage maps developed for two related populations with one common parent in order to compare the reliability of marker order and distances between markers.

In addition, different computer packages have been used for linkage analysis, e.g. Mapmaker/Exp. and JoinMap. Both use pairwise recombination rate to find marker order and to estimate the distances between marker loci. However, these two programs give somewhat different results. The linkage map for Wt 11238 (tester line) × Wt 3557 (cv. Paloma) population has been constructed using the maximum likelihood method (Mapmaker/Exp. v. 3.0) and the least squares (regression method) (JoinMap v.3.0 software). Of the markers analyzed 72% were AFLPs, 13% RAPDs, 4% morphological characters, 4% isozymes, 4% ISSRs, 2% CAPSs and 1% STSs. The results obtained from these two approaches were compared. Two versions of the map spanned 2172 cM and 841 cM respectively. The average distance between adjacent markers was 22 cM and 10 cM. The comparison was also made with the map published earlier for the Wt 10245 × Wt 11238 population (one parent in common).

Materials and methods

Plant material

Two populations from the cross Wt11238 × Wt3557 were investigated: an F₂ population of 116 plants and an F₄ population of 108 plants. The Wt3557 parental line is the cultivar Paloma and the Wt11238 (=WL1238 or NGB1238) is a tester line. Parental lines were obtained from the *Pisum* Gene Bank at Wiatrowo. The second cross involved the parental lines Wt10245 × Wt11238. The map for this population has been described by Irzykowska et al. (2) and included 204 markers and 9 linkage groups. The length of the map was determined to be 2416 cM with an average distance between adjacent markers of 12 cM.

Map construction and comparison of linkage groups

For the linkage analysis in the Wt11238 × Wt3557 populations, DNA markers (AFLP, RAPD, ISSR, CAPS, STS) as well as morphological markers (*a*, *b*, *D*, *tl*, *gp*, *cp*, *te*, *i*, *r*, *Fs*) and allozyme loci (*Acp1*, *Lap1*, *Lap2*, *Aat-m*, *Aat-p*, *Idh*, *Est2*, *Est1*, *Gal2*, *Gal3*) were used (Irzykowska et al. 2001). Goodness-of-fit to the codominant 1:2:1 or dominant 3:1 ratio was tested by the χ^2 analysis using the computer program JoinMap (11). The genetic map construction was performed by the MAPMAKER/EXP v. 3.0 software as previously described (3). The Haldane function was used.

For the population Wt10245 × Wt11238, the analysis of markers was performed as described in (2).

The reconstruction of linkage groups was made using the Join Map v. 3.0 computer program. Segregated markers were grouped and distances among them were calculated. For all linkage groups a minimum LOD score was 1.00, LOD for grouping = 3.0 or 4.0, recombination rate < 0.4, ripple value = 1, jump threshold = 5.0. The jump threshold delays the inclusion of a marker in the first two rounds to the next round, if this marker raises the χ^2 value of the lack of fit by more than the jump threshold. The pairwise recombination frequencies were estimated and the corresponding LOD values were calculated. If several estimates of the recombination frequency

between a pair of markers were available they were replaced by a single value after appropriate weighting (9). The Haldane mapping function was chosen. The “fixed order” option was taken to order the reference markers.

Maps from both populations can be compared if common and reference markers exist. The term “reference markers” stands for the markers, placed on the map, published by the *Pisum* Mapping Committee (14) and the map with expressed sequence tags (EST) (1). Segregation of these markers was observed in analysed mapping populations (Wt10245 x Wt11238 and Wt11238 x Wt3557).

The term “common markers” stands for markers revealing polymorphism in both populations. The number of common markers for the linkage groups are given in Table 1.

Table 1. Number of reference and common markers for investigated pea populations

Linkage group	Wt11238 x Wt3557		Wt10245 x Wt11238
	reference markers	common markers	reference markers
I	4	4 (2)	3
II	4	4 (2)	5 (4 in JoinMap)
III A	2	1 (1)	
III B	2	2 (2)	4
IV	1	1 (1)	3
V A	2	3 (2)	
V B	5	3 (3)	10 (9 in JoinMap)
VI A		2 (1)	1
VI B	1	-	1
VII A	1	-	
VII B	-	2	3

In parentheses: reference markers which were common.

Results and Discussion

The genetic linkage map for Wt 11238 x Wt 3557 population comprised 86 markers (100 in Mapmaker/Exp.) with average distance 10 cM between markers (22 cM in Mapmaker/Exp.) and total length 841 cM (2172 cM in Mapmaker/Exp.). The number of polymorphic markers, population description and comparison with earlier described Wt 10245 x Wt 11238 population is presented in Table 2.

Table 2. Description of pea populations used for mapping

Population Characteristic	Wt 11238 x Wt 3557		Wt 10245 x Wt 11238
	Mapmaker	JoinMap	Mapmaker
Population size		116 F ₂	114 F ₂
Total number of polymorphic markers		108 F ₄	104 F ₄
Number of linked markers		264	240
AFLP	100	86	204
RAPD	51 (9)	41 (11)	140
ISSR	23	20	24
CAPS	4	3 (1)	10
STS	4	4	5
isozymes	3	3	1
morphological	5	5	11
	10	10	13

In parentheses: markers with distorted segregation.

The population size and total marker number was similar to those from Wt10245 x Wt11238 population. However, the large number of unlinked markers was characteristic for Wt11238 x Wt3557 population (version in Mapmaker/Exp. – 56% unlinked markers, version in JoinMap – 67% unlinked markers). The described level is

higher than reported earlier (2 – 15% in pea, 7 – 19% in other legumes) (2, 5, 6, 8, 10). One of the possible reasons is the construction method for the mapping population. DNA was isolated from five randomly chosen plants of the F₄ generation, which were an offspring of each ancestor F₂ plant. DNA was combined in bulk. The observation was written down as F₂ plant data, but after 3 meiotic cycles, not after 1. The probability of recombination has been increased. The inspection in Wt10245 x Wt11238 population was made in the F₃ generation (after 2 recombination cycles), so the level of unlinked markers was lower. Random selection of 5 plants from the F₃ generation changed the assessment of the recombination frequency. Another choice in the next generation may increase this error. The high level of unlinked markers could be caused by considerable involvement of dominant markers among total analyzed markers. AFLP, RAPD and ISSR markers constituted of 94% markers.

Two individual maps for Wt11238 x Wt3557 population were generated using two programs—Mapmaker/Exp. and JoinMap. The identity of markers in both versions of the map was checked and presented in Table 3.

Table 3. The percentage of identical markers in both versions of the map for Wt 11238 x Wt 3557

Linkage group	marker number	Mapmaker	JoinMap	marker number
I	15	100 % identical markers like in JoinMap	94 % identical markers like in Mapmaker	16
II A	7	100 % identical markers		12
II B	5	100 % identical markers		9
III A	12	75 % identical markers	100 % identical markers	9
III B	8	100 % identical markers	88 % identical markers	9
IV	6	83 % identical markers	100 % identical markers	5
V A	5	100 % identical markers		16
V B	27	59 % identical markers	100 % identical markers	8
VI	7	100 % identical markers	87 % identical markers	2
VII A	4	100 % identical markers	50 % identical markers	4
VII B	4	100 % identical markers		

In most cases the same markers were included in the map in both programs. The reference marker order was consistent with this presented by the reference maps (1, 14). If any discrepancies were observed, one of the solutions was applied depending on the software used. The marker order can be changed and corrected by a user in the Mapmaker/Exp. This option is not available in JoinMap. The user is able to fix the order of markers (option “fixed orders”). However, if the order is significantly inconsistent, the marker introduction into specific group doesn't proceed (13). In some cases the “fixed orders” option had to be used regarding reference markers to keep agreement of marker order with the map published by the *Pisum* Mapping Committee and the linkage map with EST (1, 14). This option was applied in the analysis of LG I, II, III in the population Wt 11238 x Wt 3557 and LG I, II, III, V in the Wt 10245 x Wt 11238 population. The application of the option “fixed orders” can cause the reference markers to be deleted from the analysis. Such a deletion occurred for the reference marker “s” in the second linkage group of the map of population Wt 10245 x Wt 11238. The final map for Wt11238 x Wt3557 obtained in JoinMap is presented in Fig. 1. The reference markers from the map published by the *Pisum* Mapping Committee and from the linkage map with EST were marked in bold, and the markers common with Wt10245 x Wt11238 were marked in a box.

Different maps can be compared when common polymorphic markers exist. AFLP fragments obtained using the same primer combinations and displaying the same molecular weight, are generally thought to be homologous (12). Our comparison of the AFLP profiles of two mapping populations revealed 21 DNA fragments that appeared to be segregating in both populations. After construction of the Wt11238 x Wt3557 reference markers from the

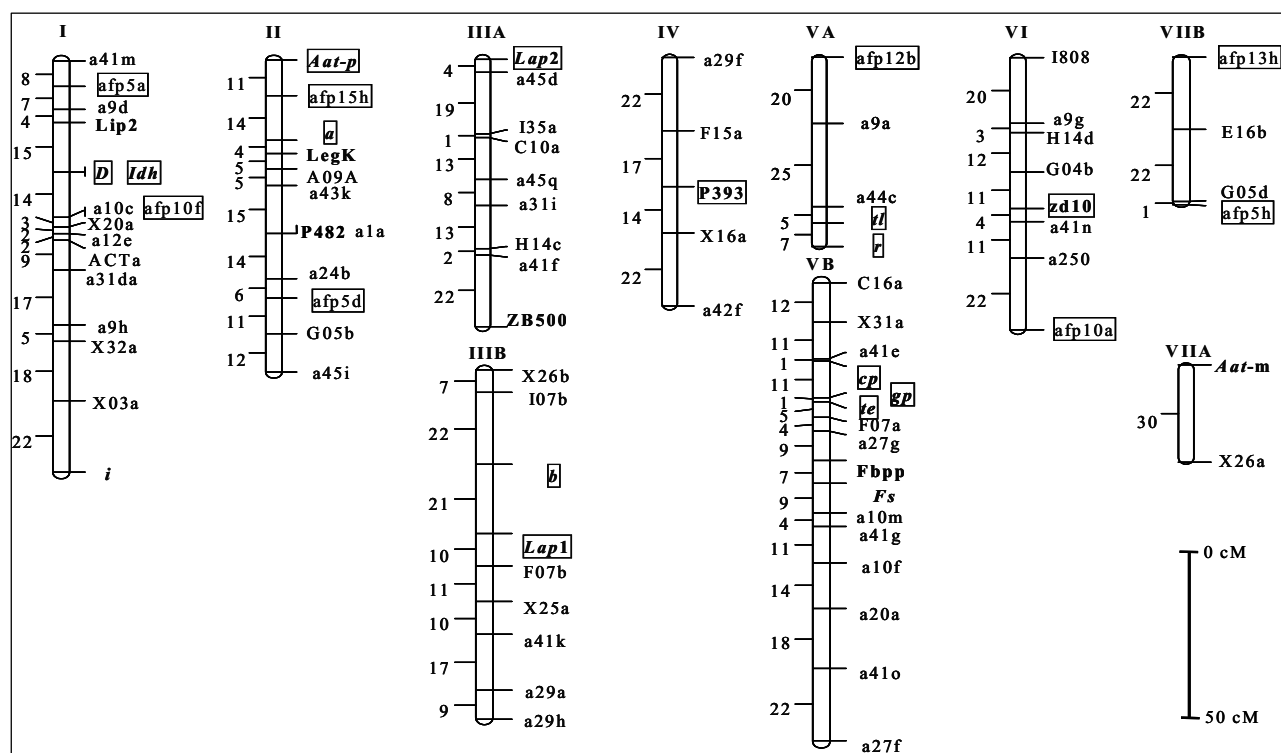


Fig. 1 The linkage map for Wt11238 x Wt3557 population (*Pisum sativum* L.).

map published by the *Pisum* Mapping Committee and from the linkage map with EST were marked in bold, and the markers common with Wt10245 x Wt11238 were marked in a box.

Different maps can be compared when common polymorphic markers exist. AFLP fragments obtained using the same primer combinations and displaying the same molecular weight, are generally thought to be homologous (12). Our comparison of the AFLP profiles of two mapping populations revealed 21 DNA fragments that appeared to be segregating in both populations. After construction of the Wt11238 x Wt3557 map and a comparison with the Wt10245 x Wt11238 map, eight of these were placed in the corresponding linkage groups in both populations. The remaining common AFLP markers did not show any linkage in Wt11238 x Wt3557 population.

The heterogeneity test was performed to allow comparison of the recombination rates between common markers. The test threshold value was $\chi^2=3.84$ for error probability $P=0.05$ and 6.63 for error probability $P=0.01$ and for 1 degree of freedom. All values were not skewed significantly except the recombination rate between marker pairs in LG II and LGV. Four common markers in LG II were arranged in six possible pairs and two of these tests were significant (a and $afp15h$ - $\chi^2=11.89$, and a and $afp5d$, $\chi^2=4.87$). Three common markers in LG VB were composed in possible pairs, and one test was significant (te and gp , $\chi^2=10.74$), reflecting a difference in marker order in the different populations ($te - gp - cp$ in Wt11238 x Wt3557 population, $te - cp - gp$ in Wt10245 x Wt11238 population). The significant value for the heterogeneity test probably was produced by an inaccuracy in one of the individual maps. Such inconsistencies make the map comparison and map possible integration more difficult.

Table 4 summarizes the results of mapping for populations Wt11238 x Wt3557 and Wt10245 x Wt11238 with Mapmaker/EXP 3.0 and JoinMap 3.0.

The lengths of individual linkage groups in Mapmaker/Exp. varied from nearly equal to 5.5 times longer compared with the analogous groups created by JoinMap. The total map length in Mapmaker/Exp. was 2.5 times longer than the total map length in JoinMap for both populations. The same mapping function was used. This dissimilarity results not only from the different number of markers in the related groups but also from the distinct calculation approach in both programs. The Mapmaker calculates the map length as the sum of adjacent

distances. This method assumes an absence of interference. JoinMap uses all pairwise estimates for calculating the total map length. The interference is taken into account (4, 7).

Table 4. The comparison of a linkage group length and marker numbers in both populations created by Mapmaker/EXP 3.0 and JoinMap 3.0

linkage group	Population							
	Wt 11238 x Wt 3557				Wt 10245 x Wt 11238			
	Mapmaker / Exp.		JoinMap		Mapmaker / Exp.		JoinMap	
length (cM)	marker number	length (cM)	marker number	length (cM)	marker number	length (cM)	marker number	
I	285	15	126	16	388	37	99	36
II A	120	7						
II B	92	5	97	12	341	30	220	31
III A	303	12	82	9				
III B	154	8	107	9	504	37	140	37
IV	122	6	75	5	256	20	83	21
V A	74	5	57	5				
V B	765	27	139	16	351	34	199	27
VI A					185	15	63	7
VI B	80	7	83	8	83	3	33	3
VII A	83	4	45	4			-	-
							36	5
VII B	94	4	30	2	276	22	75	16
total:	2172	100	841	86	2384	198	948	183

The stable map should present the final version similar and independent of the calculation approaches and the parameter settings (13). The differences in group length and marker number were observed for two versions of the map for populations Wt11238 x Wt3557 and Wt10245 x Wt11238. The reference marker order was consisted with this published (1, 14).

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